014083

PROHEXADIONE CALCIUM

Chronic oral (§83-1[b])

EPA Reviewer: Albin B. Kocialski Ph.D.
Registration Action Branch I/HED (7509C)

Work Assignment Manager: Sanjivani Diwan, PhD
Toxicology Branch 1/HED (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Chronic Toxicity Study in Dogs with Rangefinding study
OPPTS Number: 870.4100 OPP Guideline Number: §83-1b

DP BARCODE: D246707 SUBMISSION CODE: S543930
P.C. CODE: 112600 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Prohexadione calcium (91.9-93.8% a.i.)

SYNONYMS: Calcium salt of 3,5-dioxo-4-propionyl-cyclohexane-1-carboxylic acid; BX-112

CITATIONS: Wrench, S.M., McLean, T.A., Buist, D.P. (1997) BX-112 Toxicity Study in Beagle Dogs (Final Report - Repeated Daily Dosage for 52 Weeks). Huntingdon Research Center Ltd., Huntingdon, Cambridgeshire, England. Laboratory Project ID. KCI 40/911211, February 3, 1997. MRID 44457755. Unpublished

Massey, J.E., Horner, S.A., Buist, D.P. (1997) BX-112: Preliminary Oral Toxicity Study in Beagle Dogs (Final Report - Repeated Daily Dosage for 4 Weeks). Huntingdon Research Center, Ltd., Huntingdon, Cambridgeshire, England. Laboratory Project ID. KIC 29/89281, February 3, 1997. MRID 44457751. Unpublished

SPONSOR: BASF Corporation, Agricultural Products, P.O. Box 13528, Research Triangle Park, North Carolina

EXECUTIVE SUMMARY: In a chronic toxicity study (MRIDs 44457755 and 44457751), prohexadione calcium (91.9-93.8% a.i.) was administered to purebred Beagle dogs (4/sex/group) at doses of 0, 20, 200, or 1000 mg/kg/day (limit dose) by gelatin capsule for approximately 12 months. No deaths occurred during the study and no treatment-related effects were observed in the clinical appearance or behavior of dogs in any treatment group.

When compared to concurrent controls, no treatment-related differences in body weights, food and water consumption, ophthalmoscopic abnormalities, hematological or clinical chemistry parameters, organ weights, or gross pathology were observed. It was stated that no abnormalities were observed in bone marrow smears and that all smears were found to be normal in cellularity, distribution, and morphology; however, no data were submitted.

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The kidney was the target organ. When compared to concurrent controls, variations in urinalysis parameters in the 200 mg/kg animals included: increased urinary volume (↑34-90% at weeks 13 and 52) and sodium concentrations (↑24-83% at week 52) in males and females, and in the males. increased sodium (↑69-94%, at weeks 13 and 39, $p<0.05$ or 0.01) levels. Non-neoplastic microscopic findings at 200 mg/kg included: minimal dilation of basophilic cortical tubules with fibrosis in the kidneys (3/8 treated [2/8 males] vs 0/8 controls); moderate dilation of cortical tubules (1/8 treated vs 0/8 controls); and minimal dilation of basophilic cortical tubules without fibrosis (1/8 treated vs 0/8 controls).

At 1000 mg/kg, changes in urinalysis parameters included: increased urinary volume (↑36-184%, weeks 13-52, $p<0.05$ or 0.01 in the males) and sodium concentration (↑13-45%, weeks 26 and 52) in males and females, and in the males, increased sodium (↑36-113%, at weeks 13 and 39, $p<0.05$) concentrations. Despite an increase in urinary volume, sodium concentration was also increased. These relatively minor differences in increasing urinary volume with a concomitant increase in sodium concentration may be related to the microscopic changes in the kidneys. The possibility that these findings are related to treatment and are of toxicological significance cannot be excluded in the light of the histological changes observed in the kidneys; furthermore, there is no supporting evidence to suggest that these findings may be considered to be non-adverse. Non-neoplastic microscopic findings at 1000 mg/kg, moderate dilation of basophilic cortical tubules with fibrosis (2/8 treated vs 0/8 controls) and minimal dilation of cortical tubules (3/8 treated vs 0/8 controls) were observed. These observations corroborated the changes in urinalysis parameters. Similar findings were also noted in the two range-finding studies.
No neoplastic tissue was observed in dogs from any test group.

The LOAEL is 200 mg/kg/day, based on histopathological changes in the kidneys and increased urinary volume and sodium concentrations. The NOAEL is 20 mg/kg/day.

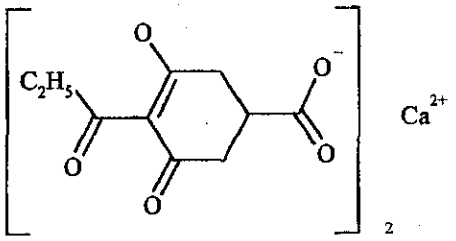
This chronic toxicity study is classified **acceptable (§83-1[b])** and <u>does</u> satisfy the guideline requirement for a chronic toxicity study in the dog.

<u>COMPLIANCE</u>: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Prohexadione calcium; BX-112
   Description: Pale yellow powder
   Lot/Batch #: G14-12 and G14-13
   Purity: 91.9-93.8% a.i.
   Stability: Not reported; it was stated that the test material "was understood to be stable for the duration of the study"
   CAS #: 127277-53-6
   Structure:



2. Vehicle: Gelatin capsule

3. Test animals: Species: Dog
   Strain: Purebred Beagle
   Age and weight at study initiation: 6 months; males, 8.1-11.3 kg; females 7.0-10.2 kg
   Source: Interfauna UK Limited, Wyton, Huntingdon
   Housing: Kennels with a floor area of 4.5 m^2, housed 2/kennel
   Diet: Standard dry diet (Diet A: Special Diets Services), 400 g/day
   Water: Tap water, ad libitum, except during overnight urine collection
   Environmental conditions:
   Temperature: 15-24°C
   Humidity: Not reported
   Air changes: Approximately 12/hour
   Photoperiod: 12 hours light/dark
   Acclimation period: 4 weeks

B. STUDY DESIGN:

1. In life dates: start: 4/26/90 end: 4/26/91

2. Animal assignment - Dogs were randomly allocated to one of the test groups shown in Table 1. Attempts were made to avoid litter effects and to minimize body weight variation.

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Table 1. Study design [a]

Test Group	Dose (mg/kg/day)	Animals Assigned	
		Male	Female
Control	0	4	4
Low	20	4	4
Mid	200	4	4
High	1000	4	4

a Control dogs received empty gelatin capsules.

3. Dose selection rationale - Dose selection was based on two subchronic oral toxicity studies in dogs. In a 13-week subchronic toxicity study (MRID 44457752) submitted with the current study, prohexadione-calcium (93.3% a.i.) was administered to beagle dogs (4/sex/dose) in capsules at dose levels of 0, 80, 400, or 2000 mg/kg/day for 13 weeks. One 400 mg/kg male displayed kidneys with moderate cortical areas of dilated basophilic tubules. When compared to concurrent controls, the 400 mg/kg animals exhibited reduced mean blood potassium levels during weeks 6 and 13 (↓7-13%). In the 2000 mg/kg group, toxicity was characterized by dilated basophilic tubules (minimal or moderate) in the cortical areas of the kidneys (4/8 treated). In addition to the dilated tubules, one high-dose male exhibited areas of minimal cortical tubular basophilia and fibrosis of the kidneys. Females excreted increased volumes of urine (↑203%, $p<0.05$) with a lower specific gravity (↓1%, $p<0.05$). In addition, blood potassium levels were decreased in both sexes during weeks 6 and 13 (males, ↓11-13%; females, ↓9-18%, respectively). The LOAEL was 400 mg/kg/day.

In a 4-week subchronic toxicity study (MRID 44457751) reviewed with the current submission, prohexadione-calcium (89.8-92.3% a.i.) was administered to beagle dogs (2/sex/dose) in capsules at dose levels of 0, 200, 600, or 2000 mg/kg/day for 4 weeks. No deaths occurred over the treatment interval. Pale and/or yellow colored feces were excreted by all 200 (6/112 possible incidences), 600 (14/112 possible incidences), and 2000 mg/kg (43/112 possible incidences) animals. The increased incidence of pale and/or yellow colored feces was dose-dependent, but was likely due to the presence of the test substance in the feces, and therefore, is not of toxicological significance. Overall (weeks 0-4) body weight gain was reduced in the 600 (↓21%) and 2000 mg/kg (↓21%, $p<0.05$) animals. When compared to concurrent controls, absolute heart (↑8-10%) and spleen weights (↑17-52%) were increased ($p<0.05$ or 0.01) for all treated groups; however, these increased organ weights were not dose-dependent and were not considered treatment-related. At the high-dose, there were decreased absolute uterus (↓44%) and testes (↓23%) weights, but these were not statistically significant. The LOAEL was 2000 mg/kg/day based on decreased body weight gain.

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Based on the results of these range finding studies, the doses shown in Table 1 were selected for the subsequent full chronic toxicity study.

4. Dosage preparation and analysis - Once a week, the required amount of test substance was inserted into gelatin capsules (5 mL capacity). Doses were based on body weights from the previous week and were administered once daily in capsules for 52 weeks. Control animals received a similar number of empty capsules as the high-dose group.

5. Statistics - Food consumption data were analyzed as totals over weekly intervals. Body weight data were analyzed using body weight gains. If the data consisted primarily of one particular value (relative frequency of the mode exceeded 75%), the proportion of animals with values different from the mode was analyzed by appropriate methods. Otherwise, the equality of means for data from the treatment groups was established using Bartlett's test of heterogeneity of variances. If significant ($p<0.01$) heterogeneity was found, the data were log transformed in an attempt to achieve less variance. If the variances of the original or transformed data were found to be equal, the data were analyzed using one-way analysis of variance (ANOVA) followed by a student t-test or by Williams' test for a dose-related response. If variances proved to be unequal following transformation, the data were analyzed using Kruskal-Wallis' test followed by the nonparametric equivalents of the t-test and Williams' test (Shirley's test). When appropriate, analysis of covariance was used instead of analysis of variance using the aforementioned sequence.

C. METHODS

1. Observations - All animals were "checked regularly" throughout each working day; on weekends and public holidays animals were checked in the morning and the late afternoon. Observations of individual animals were recorded daily.

2. Body weight - Animals were weighed once a week, prior to feeding. Body weight gains for the weekly intervals were not reported; overall (weeks 0-52) body weight gains were provided.

3. Food/water consumption - It was stated that the quantity of food left by the individual animals was recorded daily throughout the study; however, only weekly totals for the predosing and dosing intervals were reported. Water consumption was recorded for 3 weekdays during weeks 47 through 51.

4. Ophthalmoscopic examination - Ophthalmoscopic examinations were conducted on all animals once prior to treatment and during weeks 26 and 52 using an indirect ophthalmoscope following administration of a mydriatic.

5. Blood - Blood was collected from the jugular or cephalic vein from fasted animals once before dosing and during weeks 13, 26, 39, and 52. The checked (X) hematology and clinical chemistry parameters were examined.

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a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocytes
	Blood clotting measurements*	X	Cell morphology
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		
X	(Activated partial thromboplastin time)		

* Required for chronic toxicity studies.

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
		X	Total serum protein (TP)*
	ENZYMES		Triglycerides
X	Alkaline phosphatase (ALP)		
X	Serum alanine aminotransferase*		
X	Serum aspartate aminotransferase*		
X	Gamma glutamyl transferase (GGT)		
X	Ornithine carbamoyl transferase (OCT)		

* Required for chronic toxicity studies.

6. Urinalysis - Urine was collected from fasted, water-deprived animals over a period of 16 hours, once prior to the start of dosing and during weeks 13, 26, 39, and 52. The checked (X) parameters were examined in all samples.

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	Appearance (color, turbidity)	X	Glucose
X	Volume	X	Ketones
X	Specific Gravity	X	Bile pigments
X	pH	X	Haem pigments
X	Sediment (microscopic)		Nitrite
	(Protein)	X	Urobilirubin
	(Epithelial cells)	X	Sodium
	(Polymorphonuclear leucocytes)	X	Potassium
	(Mononuclear leucocytes)	X	Chloride
	(Erythrocytes)		
	(Organisms)		
	(Renal tubule casts)		
	(Other abnormal constituents)		

* Urinalysis is required for chronic toxicity studies.

7. Bone marrow examination - Bone marrow was collected from each animal by sternal puncture prior to necropsy and a smear was prepared for microscopic examination.

8. Sacrifice and pathology - After 52 weeks of treatment, all animals were sacrificed by exsanguination under pentobarbitone anaesthesia following overnight fasting. Animals were examined post mortem for gross pathology and the checked (X) tissues were collected for histopathological examination. Additionally, the (XX) organs were weighed.

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	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta* (arch and abdominal)	XX	Brain* (cerebral cortex, thalamic nuclei, mid-brain, medulla, and cerebellum)
XX	Salivary glands*	XX	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach* (body and antrum)	X	Lymph nodes* (cervical and mesenteric)	XX	Pituitary*
X	Duodenum*	XX	Spleen*	X	Eyes (optic n.)*
X	Jejunum*	XX	Thymus*		
X	Ileum*				
X	Cecum*				GLANDULAR
X	Colon*		UROGENITAL		
X	Rectum*			XX	Adrenal gland*
XX	Liver*+	XX	Kidneys*+	X	Lacrimal gland[T]
X	Gallbladder*	X	Urinary bladder*	X	Mammary gland[T]
XX	Pancreas*	XX	Testes*+ with epididymides	XX	Thyroids with parathyroids*++
		XX	Prostate		
	RESPIRATORY	XX	Ovaries*+		OTHER
		XX	Uterus*		
X	Trachea*		Cervix	X	Bone*
XX	Lungs* (with bronchi)	X	Vagina	X	Skeletal muscle*
	Nose		Oviducts	X	Skin*
	Pharynx			X	All gross lesions and masses*
	Larynx			X	Sternum

* Required for chronic toxicity studies.
+ Organ weight required in chronic studies.
++ Organ weight required for non-rodent studies.
T = required only when toxicity or target organ.

A complete complement of tissues from all animals was examined microscopically.

II. RESULTS

A. Observations

1. Mortality - No deaths occurred during the 12 month study interval.

2. Clinical signs - Incidences of pale feces occurred throughout the study in all treated and control groups. Pale feces were excreted by all 1000 mg/kg animals (8/8 treated) during 42-48/52 weeks at incidences of 0-7 days/week. At 200 mg/kg, 8/8 animals excreted pale feces during 23-29/52 weeks at incidences of 0-5 days/week. At the 20 mg/kg dose level, pale feces were observed in 8/8 animals during 5-24/52 weeks at incidences of 0-4 days/week. In the controls, pale feces were excreted by 7/8 animals during 6-12/52 weeks at incidences of 0-4 days/week. The majority of observations of pale feces occurred in the first 5 weeks in the control animals, whereas, in the treated animals incidences were

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observed throughout the study interval. The Sponsor stated and the reviewers agree that the increased incidence of pale feces was dose-dependent, but was likely due to the presence of the test substance in the feces, and therefore, is not of toxicological significance. No other effects were observed in the clinical appearance or behavior of dogs in any treatment group.

B. Body weight and weight gain - When compared to concurrent controls, no treatment-related differences in body weights were observed.

C. Food/water consumption - Food and water consumption (g/animal/day) were unaffected by treatment at all dose levels.

D. Ophthalmoscopic examination - No treatment-related ophthalmoscopic abnormalities were observed in any of the treatment groups.

E. Blood work

1. Hematology - When compared to concurrent controls, several hematological variations were noted in the 1000 mg/kg animals, such as: decreases in hemoglobin (↓7-9%) and red blood cell counts (↓9-11%) in the males and females at week 13; decreased hematocrit (↓10%, $p<0.05$), hemoglobin (↓11%, $p<0.05$), and red blood cell counts (↓12%, $p<0.05$) in the females and increased mean corpuscular volume (↑4%, $p<0.01$) in the males at week 26; decreases in hemoglobin (↓7%) and red blood cell counts (↓8%) in the females at week 39; and decreased hemoglobin (↓5%) and increased mean corpuscular volume (↑4%, $p<0.05$) in the females and decreased erythrocyte counts (↓11%) in the males at week 52. These changes in hematological parameters in the high-dose animals did not increase over the treatment period, were not dose-dependent, were minor, and/or were not statistically significant; therefore, these findings were considered not to be treatment-related.

2. Clinical chemistry - When compared to concurrent controls, several variations in clinical chemistry parameters were noted in the 1000 mg/kg animals as follows: (i) at week 13, decreases in total protein (↓5-9%, $p<0.05$), albumin (↓7-10%, $p<0.05$ or 0.01), and gamma glutamyl transferase (↑33-50%, $p<0.05$ or 0.01) in both sexes and decreased potassium levels (↓11%, $p<0.01$) in the females; (ii) at week 26, decreased total protein (↓9-10%, $p<0.05$ or 0.01), albumin (↓10%, $p<0.05$ or 0.01), and alanine aminotransferase (↓15-21%, $p<0.05$ in the males) in both sexes, decreased alkaline phosphatase (↓25%) and ornithine carbamoyl transferase (↓30%) in the males and increased chloride levels in the females (↑4%, $p<0.01$); (iii) at week 39, decreased total protein (↓7%, $p<0.01$ in the females), albumin (↓7-10%, $p<0.05$ or 0.01), gamma glutamyl transferase (↓33-50%, $p<0.05$ in the males), and potassium levels (↓4-14%, $p<0.01$ in the females), and increased phosphorus levels (↑18-31%, $p<0.05$ in the males) in the males and females; (iv) at week 52, decreased total protein (↓6-10%, $p<0.01$ in the females), albumin (↓10%, $p<0.01$), and potassium levels (↓18-19%, $p<0.01$), and increased phosphorus levels (↑4-25%, $p<0.05$ in the males) in both sexes. These changes in clinical chemistry parameters in the high-dose animals did not increase over the treatment period, were minor, and/or were not

statistically significant nor dose-dependent; therefore, these findings were considered not to be treatment-related.

F. Urinalysis - When compared to concurrent controls, variations in urinalysis parameters (Table 2) in the treated animals at week 13 included: at 1000 mg/kg, increased urinary volume in males and females (↑38-184%, $p<0.01$ in the males), and in the males, decreased pH (6.7 vs 8.1 in controls, $p<0.01$), and increased sodium (↑113%, $p<0.05$), potassium (↑83%, $p<0.01$), and chloride (↑99%, $p<0.01$) concentrations; at 200 mg/kg, increased urinary volume in males and females (↑46-90%), and in the males, decreased pH (6.8 vs 8.1 in controls, $p<0.01$), and increased sodium (↑94%, $p<0.05$), potassium (↑52%; $p<0.01$), and chloride (↑62%, $p<0.05$) levels; at 20 mg/kg, increased urinary volume in the males and females (↑35-69%) and decreased pH in males (6.9 vs 8.1 in controls, $p<0.01$).

At week 26, urinalysis variations were as follows: at 1000 mg/kg, increased urinary volume (↑36-44%) and sodium concentration (↑13-41%) and decreased specific gravity (↓1-2%, $p<0.05$ or 0.01) in both sexes, and increased pH in the females (6.5 vs 6.1 in controls, $p<0.05$); at 200 mg/kg, decreased specific gravity in males and females (↓2%, $p<0.05$) and increased pH in the females (6.5 vs 6.1 in controls, $p<0.05$).

At week 39, changes were as follows: at 1000 mg/kg, increased volume (↑69%, $p<0.05$) and sodium concentrations (↑36%, $p<0.05$) and decreased specific gravity (↓1%) in the males and decreased potassium and chloride concentrations (↓31 and 33%, respectively) in the females; at 200 mg/kg, increased sodium concentrations (↑69%, $p<0.01$) in the males.

At week 52, changes were as follows: at 1000 mg/kg, increased urinary volume (↑67-73%), pH (males, 6.4 vs 6.0 in the controls, $p<0.01$; females, 6.1 vs 5.8 in controls), and sodium concentrations (↑16-45%) and decreased specific gravity (↓1-2%, $p<0.05$) in both sexes; at 200 mg/kg, increased urinary volume (↑34-71%), sodium concentrations (↑24-83%), and pH (males, 6.3 vs 6.0 in controls, $p<0.01$; females, 6.5 vs 5.8 in controls) and decreased specific gravity (↓1-2%, $p<0.05$) in both sexes; at 20 mg/kg, increased urinary volume in the females (↑42%) and increased pH in the males (males, 6.5 vs 6.0 controls, $p<0.01$).

The minor changes in pH and the transient variations in potassium and chloride concentrations were not considered to be treatment-related. Despite an increase in urinary volume, sodium concentration was also increased. These relatively minor differences in increasing urinary volume with a concomitant increase in sodium concentration may be related to the microscopic changes in the kidneys.

Table 2. Selected urinalysis parameters.[a]

Observation	Dose (mg/kg/day)							
	Males				Females			
	0	20	200	1000	0	20	200	1000
Week 13								
Volume	77	130	146	219**	112	151	163	155
pH	8.1	6.9**	6.8**	6.7**	6.8	7.0	7.0	7.0
Specific gravity	1049	1045	1042	1041	1046	1049	1041	1039
Sodium	15.63	24.24	30.40*	33.42*	25.68	33.52	29.75	22.34
Potassium	22.64	28.47	34.40**	41.42**	30.54	39.75	38.42	27.56
Chloride	27.49	39.38	44.59*	54.63**	40.87	54.24	46.50	31.57
Week 26								
Volume	151	189	232	218	151	142	208	205
pH	6.4	6.8	6.3	6.4	6.1	6.4	6.5*	6.5*
Specific gravity	1053	1040	1036*	1043*	1060	1053	1043*	1039**
Sodium	28.22	29.55	35.80	39.76	25.87	26.01	44.35	29.32
Potassium	46.50	41.45	42.61	46.10	43.26	44.34	50.57	40.09
Chloride	53.36	44.65	59.11	59.56	48.89	49.01	63.96	42.78
Week 39								
Volume	129	173	205	218*	162	147	178	146
pH	6.5	6.8	6.5	6.5	6.4	6.6	6.7	6.8
Specific gravity	1052	1046	1041	1042	1049	1051	1041	1042
Sodium	26.46	28.98	44.61**	35.99*	31.52	27.36	33.38	23.39
Potassium	39.82	43.38	47.52	45.82	45.42	47.56	42.24	31.31
Chloride	43.06	34.63	54.50	52.67	51.58	42.95	45.20	34.81
Week 52								
Volume	131	142	176	219	97	138	166	168
pH	6.0	6.5**	6.3**	6.4**	5.8	6.1	6.5	6.1
Specific gravity	1055	1051	1040*	1040*	1060	1057	1039*	1043*
Sodium	22.25	25.15	27.63	25.86	14.26	19.16	26.03	20.65
Potassium	38.94	33.52	35.48	35.71	31.79	39.80	34.94	35.49
Chloride	38.79	31.25	37.92	34.64	28.70	35.05	32.74	31.14

a Data extracted from the study report, Tables 9a and 9b, pages 65 through 68.
* or ** Significantly different from controls at $p<0.05$ or 0.01.

G. Sacrifice and pathology

1. Organ weight - When compared to concurrent controls, the following changes were noted in the 1000 mg/kg females: decreased absolute thymus weights (↓45%, $p<0.05$) and increased relative (to body) lung weights (↑12%, $p<0.05$). In the 200 mg/kg females, decreased absolute thymus weights (↓37%, $p<0.05$) and increased relative lung weights (↑13%, $p<0.05$) were also noted.

   Two outliers were noted in the individual absolute thymus weights in the control females,

thereby, increasing the mean absolute thymus weight. Therefore, despite the statistical significance at the mid- and high-dose levels, the decreased absolute thymus weights were considered to be the result of the outliers in the control and not related to treatment. No corroborative data were found for the increased relative lung weights, and therefore, these changes were not considered to be related to treatment.

2. Bone marrow smears - It was stated that no abnormalities were observed in bone marrow smears and that all smears were found to be normal in cellularity, distribution, and morphology; however, no data were submitted.

3. Gross pathology - No treatment-related differences in gross postmortem findings were observed in the treatment groups.

3. Microscopic pathology

a) Non-neoplastic - Microscopic findings (Table 3) observed in the high-dose animals were as follows: moderate dilation of basophilic cortical tubules with fibrosis of the kidneys (2/8 treated vs 0/8 controls), minimal dilation of cortical tubules (3/8 treated vs 0/8 controls). Selected findings at 200 mg/kg included: minimal dilation of basophilic cortical tubules with fibrosis of the kidneys (3/8 treated [2/8 males] vs 0/8 controls); moderate dilation of cortical tubules (1/8 treated vs 0/8 controls); and minimal dilation of basophilic cortical tubules without fibrosis (1/8 treated vs 0/8 controls).

At 200 and 1000 mg/kg, the observations of dilated/basophilic cortical tubules with and without fibrosis were considered to be treatment-related since they corroborated the changes in urinalysis parameters and were also noted in the range-finding studies. Other microscopic changes for the high-dose animals were not correlated to changes in organ weights and gross pathological data and were also observed in the control animals; therefore, these changes were not considered to be treatment-related.

b) Neoplastic - No neoplastic tissue was observed in dogs from any test group.

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Table 3. Selected incidences and severity of microscopic findings in the kidney of dogs administered prohexadione calcium in the diet for 52 weeks. [a]

Observation	Dose (mg/kg/day)							
	Males				Females			
	0	20	200	1000	0	20	200	1000
Dilated basophilic cortical tubules and fibrosis (Total)	0	0	2	1	0	0	1	1
Minimal	0	0	2	0	0	0	1	0
Moderate	0	0	0	1	0	0	0	1
Dilated cortical tubules (Total)	0	0	1	2	0	0	0	1
Minimal	0	0	0	2	0	0	0	1
Moderate	0	0	1	0	0	0	0	0
Dilated basophilic cortical tubules without fibrosis (Total)	0	0	1	0	0	0	0	0
Minimal	0	0	1	0	0	0	0	0

a Data extracted from study report, Table 11, page 76; n=4/sex/dose.

III. DISCUSSION

A. Investigator's conclusions - The investigators concluded that prohexadione calcium administered to dogs yielded the following treatment-related alterations: an increased incidence of pale feces was observed at all dose levels; at 200 mg/kg, decreased red blood cell counts and albumin, and total protein concentrations were observed, along with, increased urinary volume, decreased specific gravity, and observations of dilated/basophilic cortical tubules in the kidneys with or without fibrosis; at 1000 mg/kg, decreased red blood cell parameters, decreased albumin, protein, and potassium levels, increased phosphorus concentrations, increased urinary volume, decreased specific gravity, and dilated/basophilic cortical tubules in the kidneys with or without fibrosis were observed. The NOAEL is 20 mg/kg/day and the LOAEL is 200 mg/kg/day.

B. Reviewer's discussion - Prohexadione calcium was administered to dogs (4/sex/group) at concentrations of 0, 20, 200, or 1000 mg/kg/day by gelatin capsule for approximately 12 months. No deaths occurred during the study and no treatment-related effects were observed in the clinical appearance or behavior of dogs in any treatment group. When compared to concurrent controls, no treatment-related differences in body weights, food and water consumption, ophthalmoscopic abnormalities, hematological or clinical chemistry parameters, organ weights, or gross pathology were observed. It was stated that no abnormalities were observed in bone marrow smears and that all smears were found to be normal in cellularity, distribution, and morphology; however, no data were submitted.

The kidney was the target organ. When compared to concurrent controls, variations in urinalysis parameters in the treated animals at week 13 included: at 1000 mg/kg, increased

urinary volume in males and females (↑38-184%, $p<0.01$ in the males), and in the males, increased sodium (↑113%, $p<0.05$) concentrations; at 200 mg/kg, increased urinary volume in males and females (↑46-90%), and in the males, increased sodium (↑94%, $p<0.05$) levels; at 20 mg/kg, increased urinary volume in the males and females (↑35-69%). At week 26, urinalysis variations at 1000 mg/kg included, increased urinary volume (↑36-44%) and sodium concentration (↑13-41%) in both sexes. At week 39, changes were as follows: at 1000 mg/kg, increased volume (↑69%, $p<0.05$) and sodium concentrations (↑36%, $p<0.05$) in the males; at 200 mg/kg, increased sodium concentrations (↑69%, $p<0.01$) in the males. At week 52, changes were as follows: at 1000 mg/kg, increased urinary volume (↑67-73%) and sodium concentrations (↑16-45%) in both sexes; at 200 mg/kg, increased urinary volume (↑34-71%) and sodium concentrations (↑24-83%) in both sexes; at 20 mg/kg, increased urinary volume in the females (↑42%). Despite an increase in urinary volume, sodium concentration was also increased. These relatively minor differences in increasing urinary volume with a concomitant increase in sodium concentration may be related to the microscopic changes in the kidneys. The possibility that these findings are related to treatment and are of toxicological significance cannot be excluded in the light of the histological changes observed in the kidneys; furthermore, there is no supporting evidence to suggest that these findings may be considered to be non-adverse.

Non-neoplastic microscopic findings observed in the high-dose animals were as follows: moderate dilation of basophilic cortical tubules with fibrosis of the kidneys (2/8 treated vs 0/8 controls) and minimal dilation of cortical tubules (3/8 treated vs 0/8 controls). Selected findings at 200 mg/kg included: minimal dilation of basophilic cortical tubules with fibrosis of the kidneys (3/8 treated [2/8 males] vs 0/8 controls); moderate dilation of cortical tubules (1/8 treated vs 0/8 controls); and minimal dilation of basophilic cortical tubules without fibrosis (1/8 treated vs 0/8 controls). At 200 and 1000 mg/kg, the observations of dilated/basophilic cortical tubules with and without fibrosis were considered to be treatment-related since they corroborated the changes in urinalysis parameters and were also noted in the range-finding studies.

No neoplastic tissue was observed in dogs from any test group.

The LOAEL is 200 mg/kg/day, based on histopathological changes in the kidneys and increased urinary volume and sodium concentrations. The NOAEL is 20 mg/kg/day.

This chronic toxicity study is classified **acceptable (§83-2[b])** and does satisfy the guideline requirement for a chronic toxicity study in the dog.

C. Study Deficiencies - The following deficiencies was noted, but will not affect the conclusions of this report:

- Bone marrow smears data were not provided.

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